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Interleukin-12 protects thermally injured mice from herpes simplex virus type 1 infection

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Abstract: Severe burn injury is associated with increased susceptibility to severe herpesvirus infections. Type 2 cytokines [interleukin (IL)-4 and IL-10] released from burn-associated CD8+ type 2 T cells (BA-type 2 T cells) have been shown to play a role in the increased susceptibility of thermally injured mice (TI-mice) to herpes simplex virus type 1 (HSV-1) infection. Because IL-12 has been shown to inhibit the generation of type 2 T cells, murine rIL-12 was injected into TI-mice exposed to HSV-1 to determine whether IL-12 could influence HSV-1 infections in individuals bearing type 2 T cells. rIL-12 improved the resistance of TI-mice or mice inoculated with T6S cells (a BA-type 2 T cell clone) against HSV-1 infection. Type 2 cytokines were detected in sera of TI-mice or mice inoculated with T6S cells (T6S-mice). However, treatment of TI-mice or T6S-mice with rIL-12 inhibited type 2 cytokine production in the sera of these mice. All TI-mice exposed to a lethal dose of HSV-1 survived when they were treated with a mixture of monoclonal antibodies (mAbs) against type 2 cytokines. Staphylococcal enterotoxin A [an interferon-γ (IFN-γ) inducer] stimulated serum IFN-γ production in TI-mice and T6S-mice treated with rIL-12, whereas no IFN-γ was produced in mice treated with saline. These results suggest that IL-12 has the potential to protect TI-mice infected with a lethal dose of HSV-1 via a shift to type 1 T cell responses from type 2 T cell responses. *J. Leukoc. Biol.* 59: 623–630; 1996.

Key Words: type 1 T cell responses · type 2 T cell responses · interferon-γ · interleukin-4

INTRODUCTION

The increased susceptibility of burn patients to various opportunistic infections has been described previously [1–5], and the mortality of thermally injured patients is mainly associated with infections rather than physical damage to the skin resulting in abnormal metabolism [6]. In addition to pathogens such as *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Escherichia coli* [7], intracellular pathogens including herpesviruses and *Candida albicans* have been identified as pathogens in thermally injured patients [2, 3]. It has been shown in recent studies that 52% of patients became infected with or have a reactivation of either latent herpes simplex virus type 1 (HSV-1) or cytomegalovirus or both [2]. Although only 21% of patients developed invasive *Candida* sepsis, over 90% of burned patients with *Candida* sepsis died [8]. The major reason for the increased susceptibility of burned patients to these pathogens appears to be a defective cell-mediated immunity as illustrated by the inhibition of the following: DTH reactions [9]; proliferative responses to mitogens or specific antigens [10, 11]; allograft rejections [12]; the production of interferon-γ (IFN-γ), which activates natural killer (NK) cells, macrophages, and CTLs [13–15]. With regard to the suppression of cellular immunity, an increased production of type 2 cytokines [interleukin (IL)-4 and IL-10] has been highlighted in recent observations [16–22]. Because infections with certain intracellular pathogens are greatly regulated by cellular responses produced by type 1 T cells [18, 19], a predominance of cellular responses toward type 2 T cells may contribute to the decreased resistance of thermally injured patients to infection from these pathogens.

Thermally injured mice (TI-mice) have been shown to be about 100 times more susceptible to HSV-1 infection [23]. TI-mice were deficient in the production of IFN-γ (a representative type 1 cytokine) and the generation of CTLs (a typical type 1 effector T cell) [13, 24], while the production of type 2 cytokines and the generation of CD8+ type 2 T cells were at higher levels [23, 25]. The increased susceptibility of TI-mice to HSV-1 infection could be transferred to normal mice by burn-associated CD8+ type 2 T cells (BA-type 2 T cells) [23]. When BA-type 2 T cells were cultured in vitro, type 2 cytokines were produced into their culture fluids 48 h after cultivation [23]. It has been shown that suppression due to BA-type 2 T cells was ex-

Abbreviations: BA-type 2 T cells, burn-associated CD8+ type 2 T cells; HSV-1, herpes simplex virus type 1; MLTR, mixed lymphocyte-tumor cell reaction; EMEM, Eagle's minimum essential medium; MNC, mononuclear cells; MSD, mean survival time in days; SEA, Staphylococcal enterotoxin A; T6S-mice, mice inoculated with T6S cells; TI-mice, thermally injured mice; FCS, fetal calf serum; mAb, monoclonal antibody; NK, natural killer; IL, interleukin; IFN-γ, interferon-γ; TNF-α, tumor necrosis factor-α.

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pressed through the activities of type 2 cytokines because the suppressor cell activity of BA-type 2 T cells was completely eliminated when these cells were treated with a mixture of monoclonal antibodies (mAbs) directed against type 2 cytokines [26]. IL-12 stimulates the production of IFN-γ from type 1 T cells and inhibits the production of type 2 cytokines from type 2 T cells [27–30]. IL-12 is able to produce a shift from type 2 T cells to type 1 T cells [27]. In the present study, therefore, rIL-12 was administered to TI-mice to attempt to regulate type 2 T cell responses due to BA-type 2 T cells and to improve the resistance of these mice to HSV-1 infections.

MATERIALS AND METHODS

Animals

Eight-week-old BALB/c mice (The Jackson Laboratories, Bar Harbor, ME) were used in the experiments. All procedures utilizing animal experiments were approved by the Animal Care and Use Committee of the University of Texas Medical Branch at Galveston (ACUC approval number: 89-03-066).

Cells, media, and viruses

CTLL-2 cells, a cell line of IL-2 and IL-4-dependent murine helper T cells [31], were obtained from the American Type Culture Collection (Rockville, MD) and serially maintained with RPMI 1640 medium supplemented with 10% fetal calf serum (FCS), 2 mM L-glutamine, and antibiotics (complete media) in the presence of 100 U/mL of rIL-2 [31]. EL-4 thymoma cells were grown in complete media. Vero cells and murine L cells (L-Galveston cells) were grown in Eagle's minimum essential medium (EMEM) supplemented with 10% FCS, 2 mM L-glutamine, and antibiotics. Complete media supplemented with 30 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid buffer and 5×10^{-5} M 2-mercaptoethanol were used for a mixed lymphocyte-tumor cell reaction (MLTR) [23, 25] and the cytokine assay using CTLL-2 cells [31]. The Indiana strain of vesicular stomatitis virus (10^8 $TCID_{50}$/mL) grown in monolayer cultures of L-Galveston cells was used for IFN titration [13]. The KOS strain of HSV-1 was propagated in Vero cells and stored at -70°C until use [32]. The titer of the virus stock solution was 1.8×10^7 PFU/mL as assayed by the plaque method on Vero cells cultured in maintenance media (EMEM supplemented with 2% FCS, 2 mM L-glutamine, penicillin, and streptomycin) [32]. This HSV-1 preparation was given intraperitoneally (i.p.) with a 4×10^4 PFU/kg dose in normal mice or 4×10^2 PFU/kg in TI-mice to produce a 1 LD_{50} dose [23].

Reagents

Murine rIL-12 was kindly provided by Dr. Stanley Wolf (Genetics Institute Inc., Cambridge, MA) and Dr. Maurice K. Gately (Hoffmann-La Roche, Inc., Nutley, NJ). The rIL-12 was diluted in phosphate-buffered saline (PBS) containing 1% FCS just before experimental use. Anti-IFN-γ, anti-IL-2, anti-IL-4, and anti-IL-10 mAbs were purchased from Pharmingen (San Diego, CA) and murine rIFN-γ, rIL-2, rIL-4, and rIL-10 were obtained from Genzyme (Cambridge, MA). Anti-CD3 mAb (Boehringer-Mannheim Biochemicals, Indianapolis, IN), anti-L3T4, and anti-Lyt 2.2 mAbs (Accurate Chemical and Scientific Co., Westbury, NY), anti-mouse-Ig antiserum (Cappel Laboratory, Cochranville, PA), and low-tox-M rabbit complement (Cedarlane Laboratories, Hoenby, Ontario, Canada) were also used in these experiments.

Thermal injury

Thermal injury was produced in mice by use of a gas flame [13]. Briefly, mice were anesthetized with pentobarbital sodium (1 mg/25 g mouse, i.p.) before being subjected to burn injury. The mice were weighed and the hair was removed from groin to axilla with electric clippers. Asbestos cloths with windows (3.5 × 2.5 cm) were pressed firmly against the shaved back, and the area was exposed for 10 s to a gas flame by use of a Fisher burner. This procedure caused a third-degree flame burn over ~30% of the total body surface area in mice with 25 g body weight [13]. Immediately after the injury, 5 mL of PBS per mouse was administered i.p. Mice anesthetized with the same amount of pentobarbital sodium, and the hair shaved, but not subject to burn injury were used as control mice.

A clone of type 2 T cells

T6S cells, a clone of burn-associated type 2 T cells, was established in this laboratory [26]. T6S cells expressed a strong suppressor cell activity in the MLTR [26]. T6S cells produced type 2 cytokines (IL-4 and IL-10) when they were cultured in vitro without any stimulation [26]. T6S cells were serially maintained in complete media supplemented with 10 U/mL of rIL-2. In some experiments 1×10^6 cells/mouse of cultured T6S cells were adoptively transferred intravenously to normal mice and designated as T6S-mice. Two hours after the cell transfer, recipient mice were infected i.p. with a 4×10^3 PFU/kg dose of HSV-1, which corresponded to a 0.1 LD_{50} in normal mice and a 10 LD_{50} dose in T6S-mice. Mice were treated i.p. with rIL-12 2 h before and 2 and 4 days after T6S cell inoculation. As described previously [23], the susceptibility of T6S-mice to infection with HSV-1 was evaluated utilizing the following criteria: (1) the mean survival time in days (MSD) of treated groups compared with that of control groups (T6S-mice treated with saline); (2) the percent survival of treated groups 25 days after the infection compared with that of control groups. The same experiment was performed two times and the results were expressed as means in Figures 1–5.

Preparation of splenic T cells

Mononuclear cells (MNC) were prepared by Ficoll-Hypaque sedimentation from spleens of TI-mice or T6S-mice, as described previously [14, 23]. To obtain whole T cells, $CD4^+$ T cells or $CD8^+$ T cells, splenic MNC (5×10^7 cells/mL) were passed through T cell columns (whole T

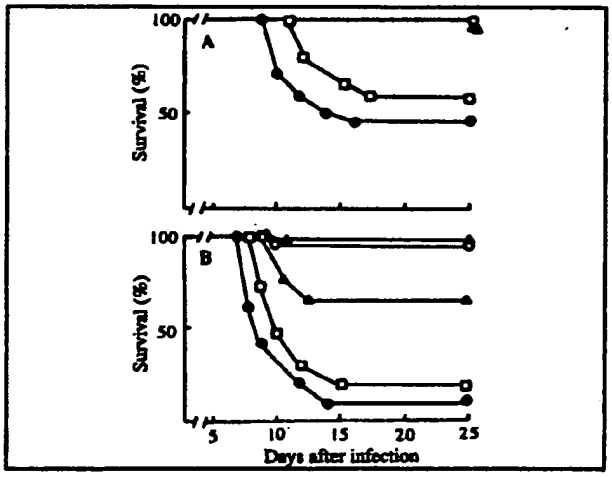

Fig. 1. Effect of rIL-12 on the HSV-1 infection in TI-mice. Mice were treated i.p. with 500 (△), 100 (▲), or 20 U/mouse (□) of rIL-12. These mice were infected i.p. with a 1 LD_{50} dose (4×10^2 PFU/kg, A) or a 10 LD_{50} dose (4×10^3 PFU/kg, B) of HSV-1. As controls, mice 1 day after thermal injury (●) and normal mice (○) were infected with the same amount of HSV-1 in each experiment. Mice were observed for 25 days after the infection to determine the number of survivors.

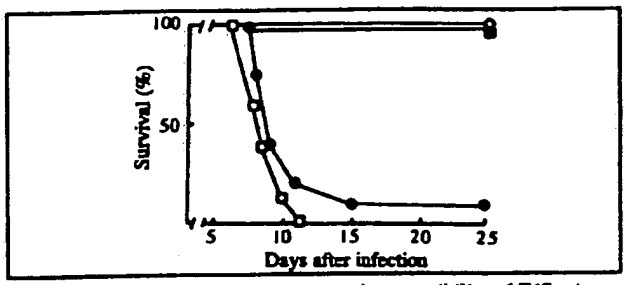

Fig. 2. Effect of rIL-12 on the increased susceptibility of T6S-mice to HSV-1 infection. Mice were treated i.p. with saline (0.2 mL/mouse, ●) or rIL-12 (500 U/mouse, ○), 2 h before, and 2 and 4 days after inoculation of T6S cells (1×10^6 cells/mouse). Two hours after T6S cell inoculation, these mice were infected i.p. with HSV-1 (4×10^3 PFU/kg). As controls, normal mice (■) or mice 1 day after thermal injury (□) were infected with the same amounts of HSV-1.

cells), CD4 subset columns ($CD4^+$ T cells), or CD8 subset columns ($CD8^+$ T cells) (R&D Systems, Minneapolis, MN) [23, 26]. When whole T cells from the column were treated with anti-Ig antiserum and complement, only a 3% reduction in viable cells was demonstrated, whereas treatment of these cells with anti-CD3 mAb followed by complement caused a 98% reduction in the number of viable cells. When $CD4^+$ or $CD8^+$ T cell fractions were treated with anti-L3T4 mAb followed by complement, 96% or 3% of viable cells were lysed. When they were treated with anti-Lyt 2.2 mAb followed by complement, 2% or 97% of viable cells were lysed, respectively. These results suggested that the purity of these three cell preparations (whole T cells, $CD4^+$ T cells, and $CD8^+$ T cells) was more than 96%.

HSV-1 infection

Mice were infected i.p. with 4×10^2 to 4×10^3 PFU/kg of HSV-1 1 day after thermal injury [23]. These amounts of HSV-1 corresponded to 0.01 to 0.1 LD_{50} in normal mice and 1–10 LD_{50} in TI-mice [23]. Two hours before and 2 and 4 days after thermal injury, rIL-12 was injected i.p. to TI-mice at doses of 20–500 U/mouse. In some experiments, anti-IL-4 mAb (200 μg/mouse), anti-IL-10 mAb (200 μg/mouse), or a mixture of both was injected i.p. to TI-mice 1 h before the infection. The effects of rIL-12 or mAbs for type 2 cytokines on the susceptibility of TI-mice exposed to HSV-1 was evaluated as described above.

Assay of suppressor cell activity

As described previously [23, 26], the suppressor cell activity of BA-type 2 T cells or T6S cells was measured in a one-way MLTR. Responders (splenic MNC from normal BALB/c mice, 5×10^4 cells/well) and stimulators (EL-4 thymoma cells, 5×10^4 cells/well) were co-cultured with or without putative suppressors (2.5×10^5 cells/well) in a 96-well round-bottomed microtiter plate for 3 days at 37°C in 5% CO_2. Before being utilized in the MLTR, all cells except responders were treated with mitomycin C, as described previously. [^{3}H]thymidine (0.5 μCi/well) was added to the plate for the last 12 h of incubation. The [^{3}H]thymidine uptake by responders was measured as previously described. The suppressor cell activity was calculated by use of the following formula: suppression (%) = [1-(cpm in the presence of putative suppressor cells/cpm in the absence of putative suppressor cells)] × 100.

Measurement of cytokine activities

Mice, subjected to thermal injury or inoculated with T6S cells, were treated i.p. with rIL-12 (500 U/mouse). Serum specimens were obtained at various days after thermal injury or T6S cell inoculation and assayed for type 2 cytokine activities [23]. For in vitro induction of IL-4 and IL-10, 2×10^6 cells/mL of splenic T cells from the above groups of mice were cultured for 24–72 h at 37°C [23]. Culture fluids harvested from cultures of these cells stimulated with anti-CD3 mAb were assayed for IL-4 and IL-10 activities. The activity of IL-2 and IL-4 was determined by the growth of an IL-2- and IL-4-dependent T cell line, CTLL-2 cells [26, 31]. Briefly, 5×10^3 CTLL-2 cells were suspended in 100 μL media and plated in 96-well plates in triplicate. Then, 100 μL of serially diluted assay samples were added to each well in the presence of either anti-IL-2 (20 μg/mL) or anti-IL-4 mAb (10 μg/mL) and plates were incubated for 18 h at 37°C in CO_2. [^{3}H]thymidine (0.5 μCi/well) was added to each well 4 h before being harvested. The incorporation of [^{3}H]thymidine into CTLL-2 cells was measured by a liquid scintillation counter. As a control, various concentrations of rIL-2 or rIL-4 were added to appropriate wells to prepare standard curves. The IL-10 activity was detected by enzyme-linked immunosorbent assay using anti-IL-10 mAb [23, 33]. For the induction of IFN-γ, mice 6 days after thermal injury or 2 days after T6S cell inoculation were stimulated with Staphylococcal enterotoxin A (SEA, 6.25 mg/kg, i.p.) [13]. Serum specimens, obtained from these mice at various times after the stimulation, were assayed for their IFN activities by means of a plaque reduction assay utilizing L-Galveston cells infected with vesicular stomatitis virus, as described previously [13–15]. The IFN titer was determined by the reciprocal of the greatest dilution of the test sample that reduced virus plaques by 50%. The IFN titer obtained was standardized to international units by use of reference murine IFN-γ (G-002-904-511). The assay was performed three times, and the results were expressed as the mean of these three tests.

Statistical analysis

Results were analyzed statistically using Student's *t*-test (MSD, suppressor cell activities, cytokine activities) and χ^2 analysis (% survival of mice exposed to HSV-1). If a P value was less than 0.05, the result obtained was considered significant.

RESULTS

rIL-12 improved the resistance of TI-mice exposed to HSV-1

In the previous studies [23] TI-mice have been shown to be 100 times more susceptible to the HSV-1 infection compared with unburned normal mice. To determine the effect of rIL-12 on the susceptibility of TI-mice to infection, three groups of 40 mice were treated with 500, 100, or 20 U/mouse of rIL-12 2 h before and 2 and 4 days after thermal injury, respectively. Twenty-four hours after thermal injury, each group was divided into two groups. One

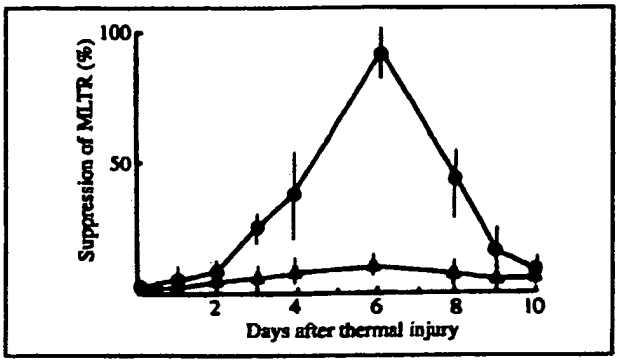

Fig. 3. The suppressor cell activity of splenic T cells from TI-mice treated with or without rIL-12. Mice were treated i.p. with saline (0.2 mL/mouse, ●) or 500 U/mouse of rIL-12 (▲). Splenic T cells were prepared from these mice various days after thermal injury and assayed for their suppressor cell activities in the MLTR

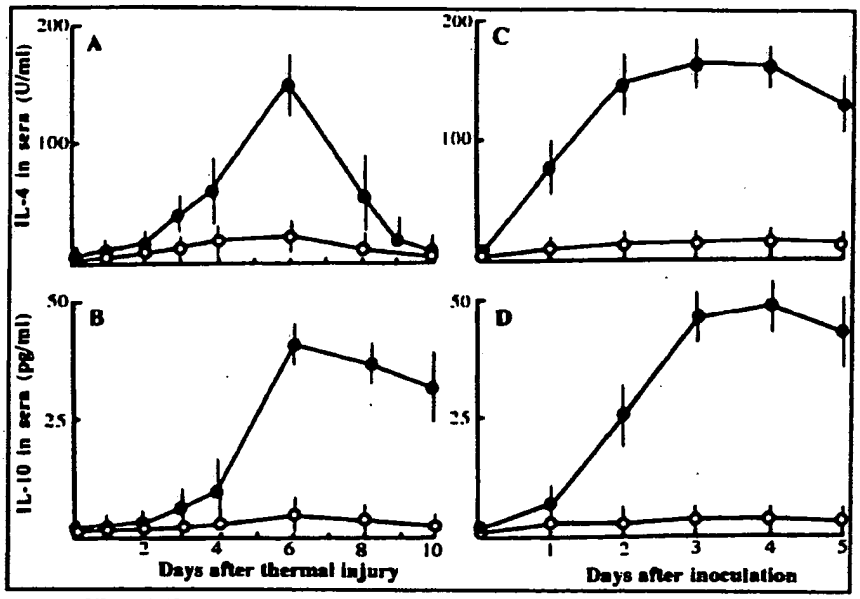

Fig. 4. Effect of rIL-12 on type 2 cytokine production in sera of TI-mice or T6S-mice. Mice were treated i.p. with saline (0.2 mL/mouse, ●) or 500 U/mouse of rIL-12 (○) (A and B). Mice inoculated with T6S cells (1×10^6 cells/mouse) were treated i.p. with saline (0.2 mL/mouse, ●) or rIL-12 (500 U/mouse, ○) (C and D). Serum specimens prepared from these mice various days after thermal injury or after the inoculation of T6S cells, were assayed for IL-4 (A and C) and IL-10 (B and D) as described in text.

group was infected with a 1 LD_{50} dose of HSV-1 and the other was infected with a 10 LD_{50} dose of HSV-1. As controls, two groups of 10 normal mice and 20 TI-mice treated with saline were exposed to a 1 LD_{50} or 10 LD_{50} dose of HSV-1, respectively. As shown in **Fig. 1A**, 50% of TI-mice treated with saline (control) died due to HSV-1 infection (MSD, >18.9 days), all normal mice and TI-mice treated with 100 or 500 U/mouse of rIL-12 survived over 25 days (MSD, >25 days). The difference in mortalities and MSD between controls and the two groups of rIL-12-treated mice was statistically significant (survival %, $P < 0.01$; MSD, $P < 0.05$). However, 20 U/mouse of rIL-12 did not produce a significant number of survivors in TI-mice (MSD, >20.4 days). When TI-mice were infected with a 10 LD_{50} dose of HSV-1, 100% of TI-mice treated with 500 U/mouse of rIL-12 (MSD, >25 days, $P < 0.001$), 70% of TI-mice treated with 100 U/mouse of rIL-12 (MSD, >22 days, $P < 0.001$), and 15% of TI-mice treated with 20 U/mouse of rIL-12 (MSD, >13.6 days, not significant) survived, respectively (Fig. 1B). All of the normal mice survived HSV-1 infection, and 95% of TI-mice treated with saline died within 14 days after the infection (MSD, 11.2 days). When viral titers in spleens of TI-mice 2 days after the infection were measured in Vero cells by a standard plaque assay, a significant reduction in the HSV-1 titer was demonstrated in groups of mice treated with 500 U/mouse of rIL-12 compared with that of control groups (data not shown). These results indicated that TI-mice exposed to HSV-1 were protected by the rIL-12 treatment, and the host-mediated anti-viral effect of rIL-12 in TI-mice depended on the amount of rIL-12 that was administered.

Effect of rIL-12 on the HSV-1 infection in T6S-mice

The increased susceptibility of TI-mice to HSV-1 infection could be transferred to normal unburned mice by inoculation with BA-type 2 T cells [23]. Therefore, the protective activities of rIL-12 on the opportunistic HSV-1 infection was examined in normal mice inoculated with T6S cells (a clone of BA-type 2 T cell). When normal mice were inoculated with 1×10^6 cells/mouse of T6S cells and exposed to a 4×10^3 PFU/kg of HSV-1 (corresponding to 0.1 LD_{50} in normal mice and 10 LD_{50} in TI-mice), all recipient mice died within 15 days after the infection (**Fig. 2**). However, all control mice inoculated with splenic T cells from normal mice survived after infection with the same amount of HSV-1. This indicated that normal mice were susceptible to HSV-1 infection after inoculation with T6S cells with the levels of susceptibility being the same as observed in TI-mice. However, 100% of T6S-mice infected with HSV-1 survived when they were treated with rIL-12 (500 U/mice; Fig. 2). These results indicated that the increased susceptibility of T6S-mice to HSV-1 infection was reversed to normal levels when these mice were treated with rIL-12.

Appearance of type 2 T cells and type 2 cytokines in TI-mice treated with or without rIL-12

Next, the generation of type 2 T cells in TI-mice treated with or without rIL-12 was examined. In this experiment mice received 500 U/mouse of rIL-12 2 h before and 2 and 4 days after thermal injury. The activity of suppressor cells, which have previously been characterized as $CD8^+$ $CD11b^+$ $TCR\gamma/\delta^+$ IL-4- and IL-10-producing T cells [23],

TABLE 1. Effect of mAbs Direct Against Type 2 Cytokines on the Survival of TI-Mice and T6S-Mice with HSV-1*

Mice	Treatment	No. of Mice	MSD	Survival (%)
Normal Mice	Saline	10	>25.0	100
TI-mice	Saline	10	11.8	0
TI-mice	Anti-IL-4 mAb	20	>22.8*	70*
TI-mice	Anti-IL-10 mAb	20	>19.5*	60*
TI-mice	A mixture of mAbs	20	>25.0*	100*
T6S-mice	Saline	10	9.2	0
T6S-mice	Anti-IL-4 mAb	20	>17.9*	60*
T6S-mice	Anti-IL-10 mAb	20	>15.5*	60*
T6S-mice	A mixture of mAbs	20	>25.0*	100*

*Normal mice inoculated intravenously with 1 x 10^6 cell/mouse of T6S cells were used as T6S-mice. Anti-IL4-mAb, anti-IL-10 mAb, or a mixture was administered i.p. to TI-mice or T6S-mice 2 h before HSV-1 infection (4 x 10^5 PFU/kg). As controls, TI-mice and T6S-mice were treated with saline and infected with the same amount of HSV-1. Mice were observed for 25 days after the infection to determine the mean survival time in days (MSD) and % survival. *$P<0.001$ compared with appropriate controls.

was first detected in mice 4 days after thermal injury and reached its peak 6 days after the injury, then gradually disappeared. However, the suppressor cell activity of BA-type 2 T cells was not detected in spleens of rIL-12-treated mice after thermal injury (Fig. 3). Subsequently, type 2 cytokine activities in sera of TI-mice treated with or without rIL-12 were measured. The sera from normal mice did not contain any IL-4 (< 10 U/mL, the day subjected to thermal injury shown in Fig. 4A), 160 U/mL of IL-4 was detected in sera of mice 6 days after thermal injury (Fig. 4A). However, IL-4 was not detected in sera of rIL-12-treated mice 2–10 days after thermal injury. Also, < 2 pg/mL of IL-10 was detected in sera of normal mice (the day subjected to thermal injury, shown in Fig. 4B) and TI-mice treated with rIL-12, whereas 30–40 pg/mL of IL-10 was detected in sera of mice 6–10 days after thermal injury (Fig. 4B). These results indicated that the treatment of TI-mice with rIL-12 resulted in the depletion of type 2 cytokines from the sera. Similar experiments were performed using T6S-mice, instead of TI-mice, in order to determine the role or the inhibitory effect of rIL-12 on the production of type 2 cytokines in sera of TI-mice. Serum specimens were obtained from mice treated with or without rIL-12 at various days after the inoculation of T6S cells and assayed for their type 2 cytokine activities as described above. As shown in Fig. 4, C and D, 150–177 U/mL of IL-4 and 20–40 pg/mL of IL-10 were measured in sera of T6S-mice 2–5 days after the inoculation. However, a significant amount of these cytokines was not detected in sera of rIL-12-treated mice on various days after the inoculation of T6S cells. These results suggest that the production of type 2 cytokines in the sera of TI-mice or T6S-mice could be regulated by the administration of rIL-12.

Effect of mAbs directed against type 2 cytokines on HSV-1 infection in TI-mice and T6S-mice

The role of type 2 cytokines, detected in sera of TI-mice or T6S-mice (see Fig. 4), on the increased susceptibility of TI-mice and T6S-mice to HSV-1 infection was studied. Anti-IL-4 mAb (200 μg/mouse), anti-IL-10 mAb (200 μg/mouse) or a mixture of both (200 μg/mouse of anti-IL-4 mAb plus 200 μg/mouse of anti-IL-10 mAb) was administered i.p. to TI-mice and T6S-mice 2 h before HSV-1 infection, and survival rates of these mice were compared with those of control mice exposed to the same amount of HSV-1. When TI-mice and T6S-mice infected with a 10 LD_{50} dose of HSV-1 were treated with anti-IL-4 mAb, anti-IL-10 mAb, or a mixture of these mAbs, 70, 60, and 100% of TI-mice and 60, 60, and 100% of T6S-mice survived over 25 days after the infection, respectively (Table 1). All infected TI-mice and T6S-mice treated with saline, however, died within 17 days (TI-mice; MSD, 11.8 days) and 14 days (T6S-mice; MSD, 9.2 days) after the infection, respectively (Table 1). These results indicated that type 2 cytokines might be released from type 2 T cells (BA-type 2 T cells and T6S cells), and play a role in the increased susceptibility of TI-mice or T6S-mice to the HSV-1 infection.

Effect of rIL-12 on the recovery of type 1 T cell responses in TI-mice

Since the resistance of TI-mice to HSV infection was improved by rIL-12 (see Figs. 1 and 2) and the production of type 2 cytokines in TI-mice was inhibited by rIL-12 (see Fig. 4), the recovery of type 1 T cell responses from type 2 T cell responses was demonstrated in TI-mice and T6S-mice treated with rIL-12. Type 1 T cell responses in TI-mice or T6S-mice treated with rIL-12 were measured by use of IFN-γ production, a representative type 1 cytokine in sera of these mice stimulated with an inducer of IFN-γ (SEA). TI-mice stimulated with SEA did not produce IFN-γ into their sera, whereas IFN-γ was produced in the sera of normal mice stimulated with SEA (Fig. 5A). However, IFN-γ (1,080 U/mL) was detected in the sera of TI-mice stimulated with SEA when rIL-12 was administered to

these mice 2 h before stimulation. A similar result was shown in T6S-mice treated with or without rIL-12 in combination with SEA stimulation (Fig. 5B). These results suggest that rIL-12 recovered the impaired type 1 T cell responses in TI-mice or T6S-mice.

DISCUSSION

Using a mouse model of thermal injury [13–15, 23], the effect of rIL-12 on the susceptibility of mice to HSV-1 infection was investigated. rIL-12 at doses of 100 and 500 U/mouse protected TI-mice exposed to HSV-1. It has been reported recently [34] that doses of more than 840 U/mouse of rIL-12 have been shown to produce toxic effects associated with the induction of tumor necrosis factor-α (TNF-α). However, a dose of 500 U/mouse of rIL-12 used in this study did not induce any detectable amounts of TNF-α in sera of TI-mice or T6S-mice (data not shown). The production of IL-4 and IL-10 in sera of mice increased following thermal injury, while the production of IL-4 and IL-10 in sera of TI-mice was not detected after treatment with rIL-12. In addition, the resistance to HSV-1 infection was improved to the levels observed in normal mice in TI-mice treated with a mixture of mAbs for type 2 cytokines. The impaired resistance of T6S-mice to HSV-1 infection was reversed by the administration of a mixture of mAbs for type 2 cytokines. The production of IL-4 and IL-10 in sera of T6S-mice was inhibited by the administration of rIL-12. SEA did not stimulate the IFN-γ production in TI-mice or T6S-mice. IFN-γ was produced by SEA in the sera of mice treated with rIL-12. These results suggest that a defect in type 1 T cell responses, reflected in decreased IFN-γ production, and enhanced type 2 T cell responses, shown by the production of type 2 cytokines and the generation of type 2 T cells, could increase the susceptibility of TI-mice to HSV-1 infection.

Based on the cytokine secretion, $CD4^+$ T cells have been classified as T helper type 1 (Th1) and T helper type 2 (Th2) cells [16–21]. In the same fashion, $CD8^+$ T cells have been separated into two different subsets: type 1 $CD8^+$ T cells and type 2 $CD8^+$ T cells [22, 35, 36]. Like $CD4^+$ T cells, type 1 $CD8^+$ T cells secrete IL-2 and IFN-γ, and type 2 $CD8^+$ T cells secrete IL-4, IL-5, and IL-10 [22, 35, 36]. Type 2 $CD8^+$ T cells were generated in various individuals bearing infections [22, 37], malignancies [38], and burn injury [23]. In our previous experiments [23], type 2 $CD8^+$ T cells were generated in spleens of mice exposed to thermal injury. The susceptibility of thermally injured mice to certain intracellular pathogens was increased by these type 2 T cells or their type 2 cytokine products [23].

A shift from type 1 T cell responses to type 2 T cell responses in immunocompromised hosts has been associated with reduced resistance to certain pathogens [16, 17]. The type 1 T cell response is an up-regulatory cellular immune response associated with increased levels of IL-2 and IFN-γ [16–21], and the type 2 T cell response down-regulates cellular responses and is accompanied by an increased production of type 2 cytokines, IL-4, and IL-10 [16–21]. Although some variability has been reported [39–41], an imbalance of the cytokine production with a shift from a type 1 T cell response to a type 2 T cell response has been associated with progression of human immunodeficiency virus infection [42, 43]. In vitro treatment of type 2 $CD8^+$ T cells, derived from patients with *Mycobacterium leprae*, with mAbs for IL-4 or IL-10 abrogated the suppressor cell activity of these T cells [44, 45]. This suggests that IL-4 and IL-10 are effector cytokines in the suppressor T cell activity of type 2 $CD8^+$ T cells. Recently, IL-13 secreted from activated Th2 cells have been included as type 2 cytokines [46]. This cytokine also has down-regulatory activities on cell-mediated immune responses [46].

It has been reported that exogenous IL-4 stimulates the generation of type 2 T cells in vitro [47, 48] and in vivo [49, 50]. Administration of anti-IL-4 mAb to mice infected with *Leishmania major* inhibited the generation of type 2 T cells that suppress the appearance of IFN-γ mRNA [49]. On the other hand, the resistance to certain infections has been shown to be associated with the increased production of type 1 cytokines [16, 17, 51]. Macrophage functions, DTH responses, the production of both complement-fixing and opsonizing antibodies and activities of CTLs and NK cells have been activated or stimulated by type 1 cytokines [16, 17, 51]. Several recent studies [27–29] have suggested that IL-12 is a stimulator of the differentiation of type 1 T cells and an inhibitor of type 2 cytokine produc-

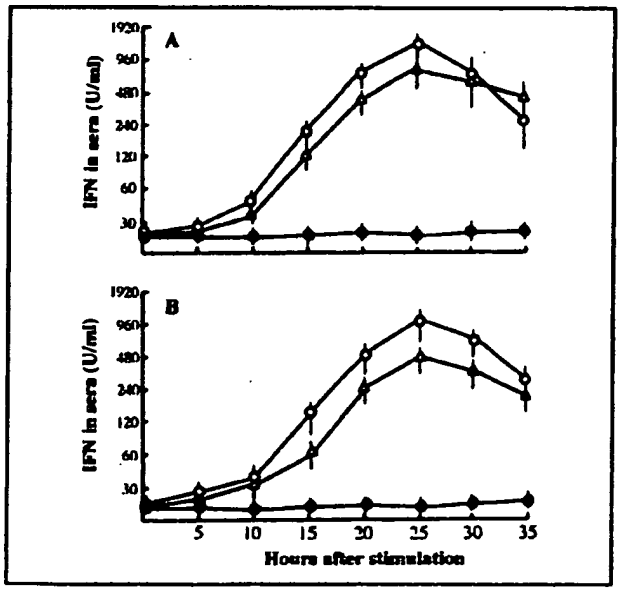

Fig. 5. Effect of rIL-12 on the production of IFN-γ in sera of TI-mice or T6S-mice. TI-mice (A) or T6S-mice (B) were treated with saline (●, 0.2 mL/mouse) or rIL-12 (500 U/mouse, △) or after inoculation with T6S cells. Six days after thermal injury (A) or 2 days after the inoculation of T6S cells (B), these mice and normal control mice (○) were stimulated with SEA (6.25 mg/kg, i.p.) to induce IFN-γ production. Serum specimens, obtained from these mice at various hours after the stimulation, were assayed for IFN, as described in text.

tion from type 2 T cells. Exogenous IL-12 induced the differentiation of type 1 cytokine-producing cells from T cell populations containing large numbers of type 2 T cells [27]. IL-12 treatment of mice immunized with *Schistosoma mansoni* resulted in suppression of mRNA production of type 2 cytokines [52]. Therefore, certain infections occurring in thermally injured patients cause a generation of type 2 T cells and production of type 2 cytokines, and IL-12 may have potency to regulate these T cell responses.

It has been demonstrated that IL-12 inhibited disease progression in leishmaniasis when administered at the time of infection, but not 1 week after infection [53, 54]. These results suggest that IL-12 is ineffective in the presence of an ongoing type 2 T cell response [53, 54]. In our preliminary studies, thermally injured mice infected with a lethal amount of HSV-1 were treated with various schedules and doses of IL-12. When thermally injured mice were treated with a 500 U/mouse dose of IL-12 1 day before HSV-1 infection, significant protective effects of IL-12 were demonstrated. When the same dose of IL-12 was administered to thermally injured mice every day for 1 week beginning 1 day after the infection or 2 and 4 days after the infection, mortality of these mice was not changed compared with control mice treated with saline. When thermally injured mice were treated i.p. with the same amount of IL-12 1 day before, and 1 and 3 days after the infection (2 h before, and 2 and 4 days after thermal injury), the maximum protective activity of IL-12 was demonstrated. Therefore, in this experiment thermally injured mice infected with HSV-1 were treated with IL-12 prophylactically and therapeutically.

In conclusion, in this experiment IL-12 was shown to lead a shift from type 2 T cell responses to type 1 T cell responses in thermally injured mice and to improve the resistance of these mice against HSV-1 infection.

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